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The role of complement in biomaterial-induced inflammation

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Abstract

Biomaterials are regularly used in various types of artificial tissues and organs, such as oxygenators, plasmapheresis equipment, hemodialysers, catheters, prostheses, stents, vascular grafts, miniature pumps, sensors and heart aids. Although progress has been made regarding bioincompatibility, many materials and procedures are associated with side effects, in particular bioincompatibility-induced inflammation, infections and subsequent loss of function. After cardiopulmonary bypass, coagulopathies can occur and lead to cognitive disturbances, stroke and extended hospitalization. Hemodialysis is associated with anaphylatoid reactions that cause whole-body inflammation and may contribute to accelerated arteriosclerosis. Stents cause restenosis and, in severe cases, thrombotic reactions. This situation indicates that there is still a need to try to understand the mechanisms involved in these incompatibility reactions in order to be able to improve the biomaterials and to develop treatments that attenuate the reactions and thereby reduce patients' discomfort, treatment time and cost. This overview deals with the role of complement in the incompatibility reactions that occur when biomaterials come in contact with blood and other body fluids.

Keywords: Complement; Biomaterial; Coagulation; Inflammation; Cross-talk

1. The complement system

The complement system (Fig. 1) consists of approximately 30 plasma and membrane-bound proteins (receptors and regulators) with a primary function of purging foreign substances, including microorganisms, foreign bodies and apoptotic cell debris from the body (Morikis and Lambris, 2001; Sahu and Lambris, 2000). It has the unique ability to discriminate "non-self" structures from "self" structures (Atkinson and Fairies, 1987).

The complement system destroys and removes substances, either by direct lysis or by mediating leukocyte function in inflammation and innate immunity. The main event in the activation of complement is the enzymatic cleavage of C3 into C3b and C3a. Activation is achieved by two enzyme complexes, the C3 convertases (C4b,2a and C3b,Bb, respectively), which are assembled by three different activation pathways. The classical pathway (CP) is triggered by the formation of antigen–antibody complexes but can also be triggered by bound C-reactive protein (CRP) and "self-structures" released from damaged cells. The mannose-binding lectin (MBL) pathway (LP) is usually activated independently of antibodies and is triggered by the binding of MBL or ficolins to specific carbohydrates, in particular those on the surface of microorganisms. Recently, an IgA-dependent activation of LP has been proposed (Roos et al., 2001). The alternative pathway (AP) is triggered directly by foreign surfaces, such as man-made biomaterials, that do not provide adequate down-regulation of the convertase.

The nascent C3b molecule has the specific biological property of binding to proteins and carbohydrates via free hydroxyl or amine groups, forming covalent ester and amide bonds, respectively. The amide bond is stable, but the ester bond is labile because of the esterolytic activity of C3b. The covalent bond is essential for the biological activity of C3b. the AP also serves as a major amplification loop of activation via the other pathways, by which an initial weak stimulus may be markedly enhanced: The AP has recently been shown to contribute to more than 80% of the C5a and C5b-9 formation induced by an initial specific activation of the CP system (Harboe et al., 2004). In addition,

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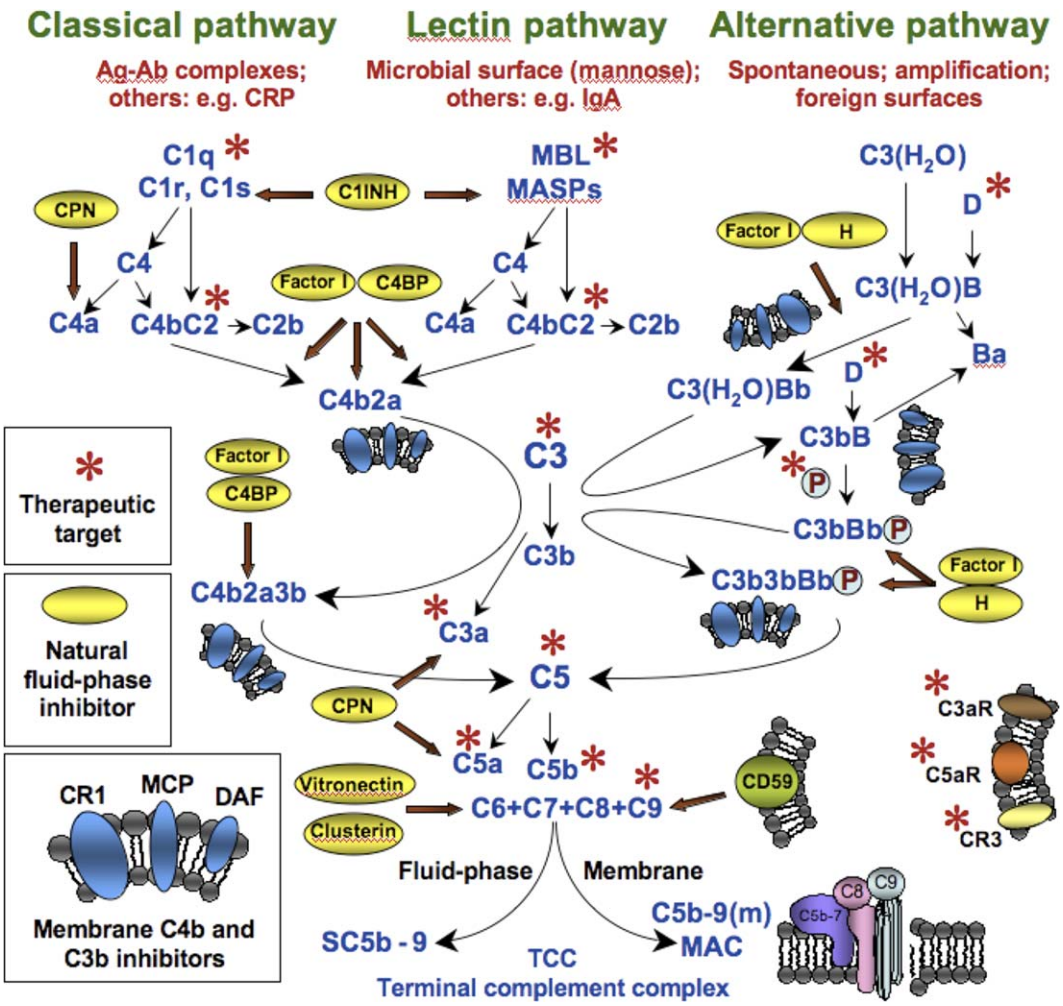

Fig. 1. Overview of the complement system, with emphasis on activation pathways, potential therapeutic targets and membrane- and fluid-phase inhibitors.

both IgG and MBL have been shown to activate complement in a C2-independent manner by bypassing the CP convertase (Selander et al., 2006; Wagner et al., 1999).

The mechanisms by which complement is activated are still not fully understood, and it should be emphasized that many of the experiments leading to our current knowledge have been performed using diluted sera and non-physiologic conditions.

Given the complexity of the activation process, there are several control points to consider for regulation. Natural regulation in vivo is provided by both plasma and membrane proteins. The primary plasma protein inhibitors are factor H and C4b-binding protein, both of which can exhibit affinity for glycosaminoglycans (Sahu and Pangburn, 1993). The principal regulatory membrane proteins located on cell surfaces are complement receptor 1 (CR1; CD35), decay-accelerating factor (DAF; CD55), membrane cofactor protein (MCP; CD46), and CD59. All of these proteins except CD59 belong to a family of proteins (regulators of complement activation, RCA) encoded by a gene cluster on chromosome 1. The proteins contain varying numbers of globular homologous short consensus repeats (SCRs). Linked together the SCRs form a "string of pearls." Each SCR consists of 60 amino acid residues, with folding of the polypeptide chain produced by two disulfide bonds (Hourcade et al., 2000). The RCA proteins prevent the release of the anaphylatoxic peptides C3a and C5a by inhibiting the C3 and C5 convertases, by promoting dissociation of the multisubunit complexes and/or by inactivating the complexes by proteolysis catalyzed by factor I. CD59, on the other hand, prevents the final assembly of the membrane C5b-9 complex. Additional fluid-phase regulators are the Cl inhibitor, which control CP and LP activation, and the anaphylatoxin inhibitor, which inactivates C3a and C5a by splitting off the C-terminal arginine residue.

Although complement is an important line of defense against pathogenic organisms, its inappropriate activation may lead to host cell damage. Complement has been implicated in several disease states, and direct evidence for its involvement has been obtained by examination of tissues from the affected patients and from animal models of disease. The tissue injury that results from complement activation is mediated directly by the membrane attack complex, C5b-9, and indirectly by the generation of anaphylatoxic peptides, C3a and C5a, which induce damage through their effects on monocytes/macrophages, PMNs, and mast cells. Furthermore, iC3b is an important ligand for CD11b/CD18 (CR3); through their interaction, activated leukocytes are attached to surface-bound iC3b. A novel iC3b complement receptor on liver Kupffer cells, termed CRIg, was recently

shown to be essential for the clearance of microbes from the portal blood (Helmy et al., 2006).

2. Mechanisms by which complement is activated on an artificial surface

2.1. Involvement of activation pathways and the deposition of C3

Many researchers have reported that exposure of biomaterial surfaces to plasma or blood either in vitro and in vivo can trigger the AP as demonstrated by generation of AP activation products (Chenoweth, 1987; Hed et al., 1984). Some studies have also demonstrated CP and/or LP activation. Lhotta et al. (1998) have shown that hemodialysis in patients with C4 deficiency leads to activation of complement, as a result of contact between the biomaterial and the patients' plasma, but the activation was much slower than in plasma from normal individuals. This finding suggests that the biomaterial can trigger a rapid activation by the CP and/or LP (C4-dependent), followed later by AP activation.

It is well established that different biomaterial surfaces have different complement-activating properties. Physical properties such as hydrophobicity and hydrophilicity are parameters that affect the activating ability. Hydrophobic surfaces are more potent activators than hydrophilic ones, and incorporation of chemical groups such as NH_3, OH or COOH influences the activation of complement (Ekdahl et al., 1993). Biomaterial surfaces with free OH and NH_3 groups are generally regarded as more prone to activate complement than others, since these groups are essential for the covalent binding of C3b (Chenoweth, 1987). However, these studies have not taken into account the fact that plasma proteins immediately bind to and cover the surface of a biomaterial surface once it has made contact with blood plasma or other body fluids. We have shown that the adsorbed C3 in this initially adsorbed protein layer is able to generate an initiating C3 convertase (Andersson et al., 2002). This reaction can occur because the adsorbed C3 is conformationally changed into a configuration that mimics that of bound C3b. This initial convertase can then trigger further activation and amplification via the AP.

2.2. The gas–fluid surface

Another type of surface that alters the configuration of C3 is the gas surface that is necessary for oxygenation of the blood in the oxygenator of the cardiopulmonary bypass (CPB) circuit. We have demonstrated that contact between the surface of the gas (oxygen, nitrogen or air) and whole blood leads to activation of complement in vitro and in vivo (Ekdahl et al., 1992; Pekna et al., 1993). This activation is a result of the generation of iC3 (hydrolyzed C3 with a C3b-like conformation) at the interface between fluid and gas. The mechanism is probably similar to that occurring on the material's surface. However, the iC3 generated on the gas surface is immediately displaced into the fluid phase and is able to generate a soluble C3 convertase. This convertase generates C3a but not soluble C5b-9 complexes (sC5b-9). These studies have demonstrated that absorbed proteins may expose sites that are specific for the activated protein, and they point to a mechanism by which other proteins such as IgG may be activated when they come in contact with material surfaces.

2.3. Methods for studying solid-phase/protein interactions and protein deposition

The interactions with human serum and plasma can be studied by using techniques that detect changes in the molecular mass associated with a surface. These include quartz crystal microbalance with dissipation (QCM-D), ellipsometry and plasmon resonance (SPR). Employing these techniques, we and others (Andersson et al., 2005; Tengvall et al., 2001; Wettero et al., 2002) have shown that when plasma proteins are deposited onto a polystyrene surface in contact with undiluted human serum under conditions in which complement activation is totally blocked, the passive adsorption of proteins occurs much more rapidly than does the active binding of proteins mediated by complement activation in serum without additives (a relatively slow and continuous process; Fig. 2). Experiments using QCM-D have revealed that the protein deposited in the absence of complement has a surface concentration of 970 ng/cm^2. Assuming a density of 1200 kg/m^3, a value between those of water (1000 kg/m^3) and protein (1400 kg/m^3), we calculated an average thickness of about 8 nm. These values obtained for the rate of binding and the thickness of the adsorbed protein layer are in good agreement with previously published data obtained by ellipsometry for undiluted serum (Wettero et al., 2002). Provided that the proteins are not deposited in clusters, this value corresponds to at least a monolayer of proteins of the size of the most common proteins in serum (albumin, 4 nm; IgG, 8 nm). Complement activation added approximately 25% to the initially adsorbed protein mass after 60 min of incubation; this layer of

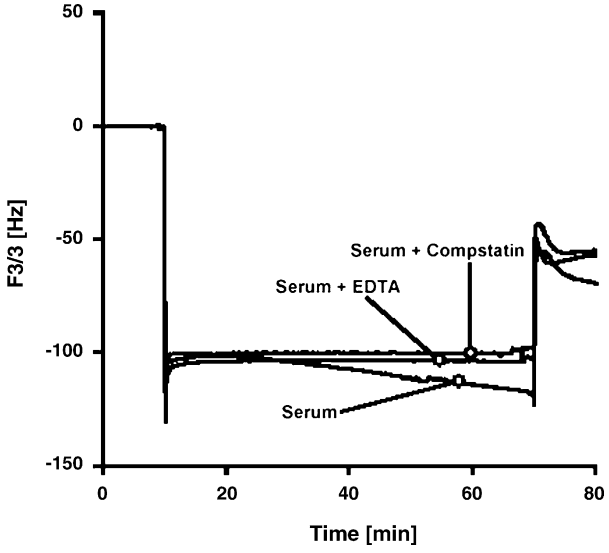

Fig. 2. Adsorption of serum proteins to polystyrene, as monitored by QCM-D. The surface was initially exposed to veronal buffered Ssline, pH 7.4, containing Mg^{2+} and Ca^{2+}. Then serum without additions, serum containing 10 mM EDTA, or 40 μM of Compstatin was added. The incubation was stopped after 70 min by exchanging serum for buffer in the chamber (Andersson et al., 2005).

complement fragments probably consists mainly of iC3b (see below).

These findings strongly suggest that complement activation occurs on top of a layer of plasma proteins. The most abundant of these proteins, albumin (42 mg/ml), IgG (11 mg/ml), and fibrinogen (3 mg/ml), have all been reported to bind to biomaterial surfaces.

2.4. C3 interaction and activation by bound serum proteins

Our in vitro binding analyses have led us to hypothesize that C3b, a prerequisite for AP activation, binds covalently to plasma proteins such as albumin, IgG and fibrinogen. The ability of a specific plasma protein to act as an acceptor for the covalent binding of C3b was assessed by assembling AP convertases on plasma protein-coated polystyrene surfaces in a QCM-D chamber (Andersson et al., 2005); AP convertases were assembled by the sequential addition of the AP components B, D, and C3.

As shown in Fig. 3, functional convertase complexes were formed on deposited IgG and human serum albumin, suggesting that these molecules can covalently bind C3b. Fibrinogen, however, did not display this property (Andersson et al., 2005). Results of a previous study have shown that the less abundant C3 and C3b molecules can also act as acceptors for C3b binding (Andersson et al., 2002).

In vivo studies have indicated that no CP activation occurs when patients with C4 deficiency undergo hemodialysis (Lhotta et al., 1998). In these patients, the initiation of complement activation is delayed, but it finally reaches the same amplitude as in patients with normal levels of C4, suggesting that only the AP is operative. In our experience, however, sera and plasma from MBL-deficient individuals show normal activation patterns

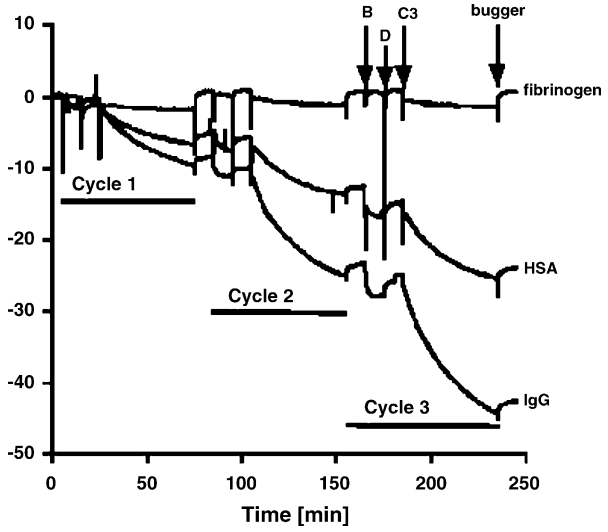

Fig. 3. Generation of AP convertases on polystyrene coated with human serum albumin or IgG, as monitored by QCM-D. For each experiment, the AP was assembled by sequentially adding factor B, factor D, C3 and buffer in three identical cycles. Each bar in the figure represents one cycle. All steps were carried out in PBS containing 1 mM Ni^{2+} to stabilize the convertase (Andersson et al., 2005). The *y*-axis represents decrease in frequency (Hz) which is proportional to the mass of the deposited proteins.

(unpublished data). These findings suggest that the C1 complex or, more likely, IgG adsorbed to the biomaterial surface in the hemodialysis device is able to trigger CP activation, a conclusion that is supported by our present study and by previous in vitro studies demonstrating that IgG adsorbed to surfaces mediates a vigorous activation and binding of C3b to the surface (Nilsson, 2001; Tengvall et al., 2001).

In our kinetic studies of uncoated polystyrene and undiluted serum to which we added the complement inhibitory peptide Compstatin (Sahu et al., 2000) to block the AP, the CP was only active for a short time, after which the C3b deposition leveled off. One explanation for this observation comes from earlier studies demonstrating that during initial complement activation, C3b binding seems to physically shield the underlying proteins, including C1q and IgG, and may also block available acceptor sites for the binding of C4b (Nilsson, 2001; Tengvall et al., 1998), thereby halting further activation via the CP. However, this limited CP activation might also reflect transient conformational changes in IgG caused by the adsorption to and denaturation of the molecules at the surface. The IgG molecules would thus be active for only a limited time. The results we obtained for serum diluted in the presence of Compstatin tend to support the second explanation: The packing density, and therefore the conformation of the IgG molecules on the surface, could explain the concentration dependence of the CP activation in these experiments. Even though the CP only deposits minute amounts of C3b on the surface, the deposition is rapid and sufficient to instantaneously trigger the AP amplification loop. We conclude that the combined sequential activation of the two pathways results in the total deposition of C3b molecules on the surface.

2.5. Covalent binding of C3

In order to confirm that C3b covalently binds to an adsorbed layer of plasma proteins, we incubated undiluted serum in polystyrene tubes and, after washing, eluted the bound plasma proteins with 2% SDS. The samples were then subjected to SDS-PAGE, followed by Western blotting using anti-C3c antibodies. By using non-reducing conditions and room temperature, covalent ester bonds could be maintained. This analysis demonstrated four distinct C3 bands, of which a significant portion of the C3 fragments had a molecular size greater than that of monomeric C3. One band most likely corresponded to monomeric C3 or C3b, while the other three had higher molecular sizes, indicating that C3 was bound to other molecules. Since the electrophoresis was performed under denaturing conditions, this binding was most likely covalent, i.e., to plasma proteins adsorbed to the polystyrene surface. After reduction with mercaptoethanol, only the β-chain and, faintly, the 40-kDa polypeptide chain of C3c were seen. This result indicates that the surface-bound C3 fragment was iC3b.

These experiments suggest that iC3b is bound to the surface-adsorbed plasma proteins via covalent bonds. The instability of the ester bond does not rule out the possibility that a larger number of molecules were originally ester-bonded to plasma proteins.

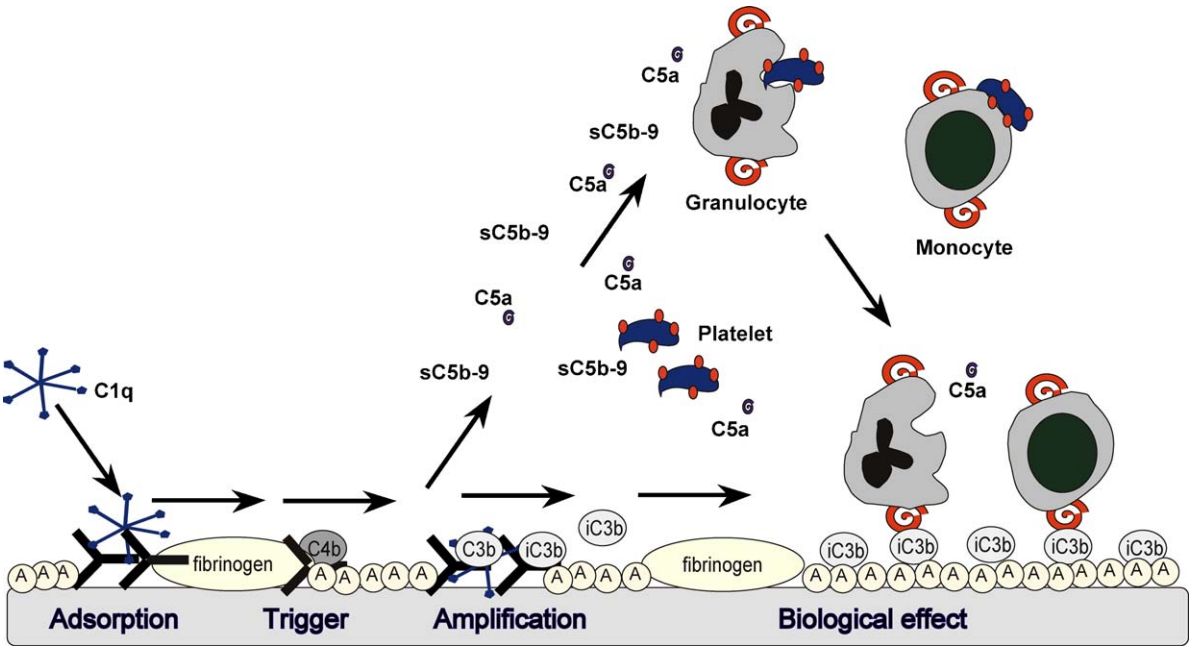

Fig. 4. Model of complement activation and iC3b binding to a biomaterial surface. Activation is triggered by molecules of the initially adsorbed protein film, which consists of immunoglobulins, fibrinogen and human serum albumin (A). Generated C5a and sC5b-9 recruit and activate platelets as well as granulocytes and monocytes, which bind to the protein-bound iC3b.

2.6. A model for C3 activation on a biomaterial surface

Taken together, these findings have allowed us to construct a model to explain how complement activation and C3b binding take place on a biomaterial surface (Fig. 4). The initiation of complement activation, which provides the initial C3b molecules that bind to the plasma protein coat on the material surface, is probably triggered by molecules of the initially bound protein film. Adsorbed IgG has been shown to initiate the CP (Nilsson, 2001; Tengvall et al., 2001), and we have demonstrated that adsorbed and conformationally changed but not cleaved C3 is able to serve as a nucleus for an initiating AP convertase (Andersson et al., 2002). Once C3b is generated and covalently bound to the protein coat, the AP amplification loop can be triggered. This amplification loop then generates the majority of the C3b molecules that bind to the material surface. Important consequences of this model, therefore, are that initiation of the AP amplification loop can be triggered by either CP, LP, or AP convertases and that a biomaterial surface that binds low levels of plasma proteins, such as a hydrophilic surface, will also be a poor activator of complement; both of these predictions have been verified empirically (Ekdahl et al., 1993).

3. The role of complement in adverse reactions induced by biomaterials in contact with blood

3.1. Cross-talk between components of the cascade systems, leukocytes and platelets

Most biomaterials, including those that are implanted into soft and hard tissues (dental implants, protheses), come in contact with whole blood when blood is present during the implantation procedure. It is therefore reasonable to assume that the inflammatory reaction against the material is greatly influenced by the initial contact with the blood, whose coagulation, contact and complement systems are the major activators of cellular elements (Fig. 5). Similar inflammatory reactions have been reported in response to the administration of micelles and other particles used for drug release applications (Szebeni, 2005; Szebeni et al., 2001).

The plasma cascades comprising the complement, coagulation, fibrinolysis and kallikrein/kininin systems act by similar principles. Although these cascades are specialized for different purposes, direct interactions between these systems have long been known to occur (Kazatchkine and Jouvin, 1984; Nydegger and Suter, 1984; Sundsmo and Fair, 1983). Furthermore, there is tight cross-talk between the cascade systems and the platelets and leukocytes in the induction of clotting and inflammation (Gorbet and Sefton, 2004).

On the protein level, coagulation enzymes have been shown to activate C3 and C4 (Kirschfink and Borsos, 1988) and to release a monocyte chemotactic activity from factor H (Ohtsuka et al., 1993). Furthermore, factor XIIa has been shown to induce the CP by activating C1r (Ghebrehiwet et al., 1983). Using a system in which islets of Langerhans are incubated with AB0-compatible whole blood in a modified version of the Chandler loops (see below), we have demonstrated that activation of the coagulation system induces the generation of C3a. This generation can be completely counteracted by the specific thrombin inhibitor melagatran (Ozmen et al., 2002).

The central role of complement as a mediator to recruit and activate leukocytes has been known for decades. It has also been known for a very long time that sC5b-9 complexes interact with

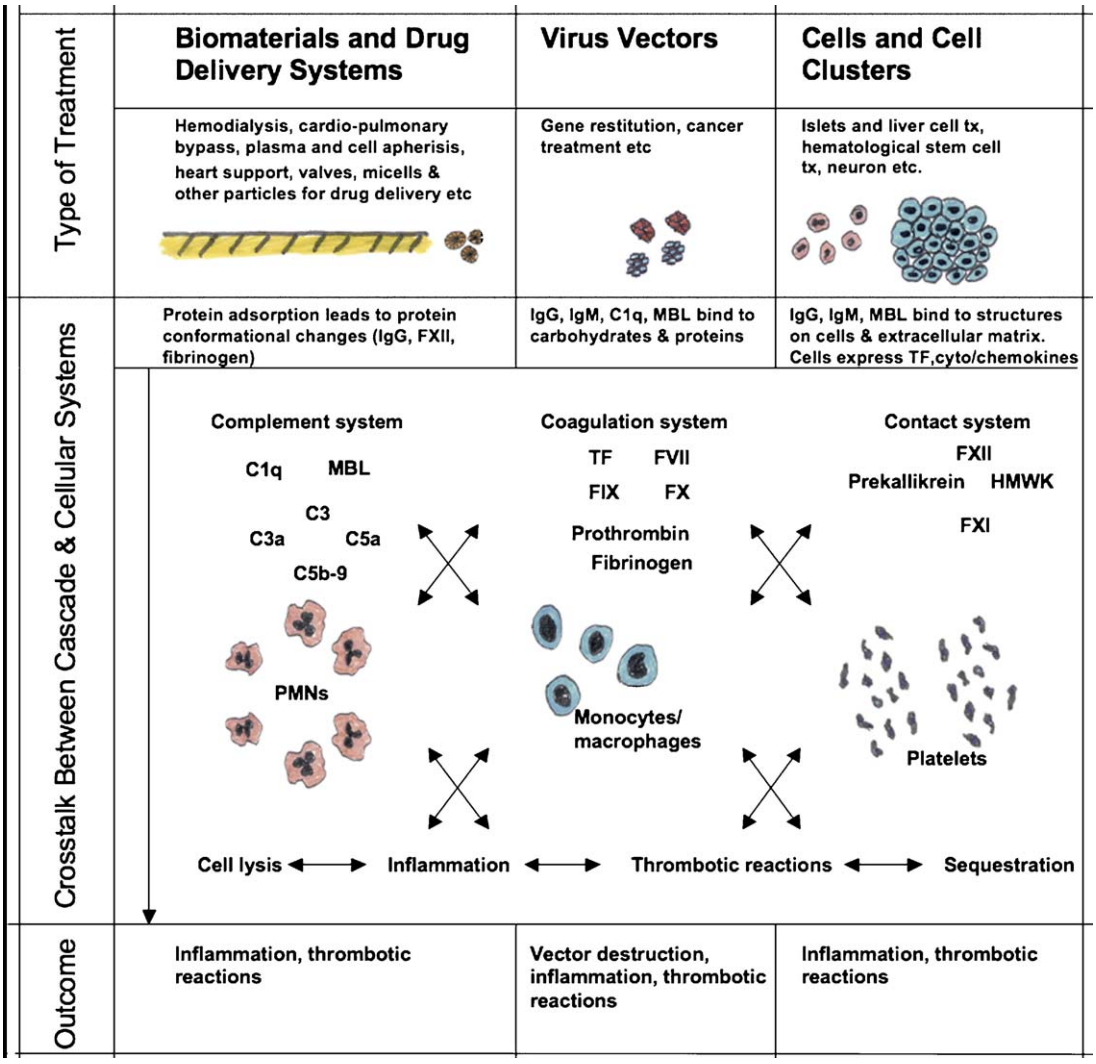

Fig. 5. Overview of the crosstalk between the cascade and cellular systems of the blood in different established and emerging treatment modalities. Top panels: types of treatment and specific examples; middle panels: recognition mechanisms by the cascade systems, and crosstalk triggered by the different recognition molecules; lower panels: the possible resulting adverse effects triggered by the treatment modalities. HMWK: High Molecular Weight Kalllikrein.

and activate platelets (Polley and Nachman, 1979), and that this activation is independent of GP IIb-IIIa, the fibrinogen receptor (Ando et al., 1989). The role of complement in platelet activation is currently being investigated further by several groups, as reviewed in Karpman et al. (2006).

In addition, both C5a and sC5b-9 complexes have been found to induce tissue factor activity in human umbilical vein endothelial cells (HUVECs) (Ikeda et al., 1997; Tedesco et al., 1997). Recently, inhibition of C5a was shown to attenuate changes in a number of coagulation markers (APTT, PT, fibrinogen, FVII:C, antithrombin, TAT and D-dimer) as well as fibrinolytic activity (plasminogen, t-PA and PAI) during experimental sepsis (Laudes et al., 2002). However, all these studies have made using of a variety of in vitro systems, and thus the physiological relevance of their observations remains unclear.

More recently, a novel mechanism of complement activation was suggested by the observation that in C3 knockout mice, thrombin can substitute for the absent AP convertase to activate C5 to functionally active C5a (Huber-Lang et al., 2006). These data suggest that complement activation is an early and primary event in both the inflammatory and thrombotic/thrombolytic pathways and that the coagulation system participates actively in this cross-talk.

3.2. Models for blood–biomaterial interactions

Since the various cascade systems and cell types in the blood are known to interact, the true effect of a biomaterial surface on the blood can only be known when whole blood is used for interaction studies (Gorbet and Sefton, 2004). We currently make use of two in vitro systems, the Chandler loop and the chamber model, to study the sequence of events that occur when biomaterial surfaces come into contact with whole blood. In order to minimize the inhibition of complement and coagulation, we use either low amounts of heparin (less than 0.5 IU/mL), or specific thrombin inhibitors such as melagatran or lepuridin (Lappegard et al., 2004a; Mollnes et al., 2002; Ozmen et al., 2002).

The Chandler loops consist of pieces of tubing filled with blood; these sections of tubing are closed into loops by means of heparinized metal connectors and allowed to rotate at 37 °C in a water bath. Using this model we have documented that various biomaterials behave differently with respect to activation of complement on the solid phase and in the fluid phase, and both phases therefore need to be considered when evaluating complement activation by biomaterials (Gong et al., 1996; Mollnes et al., 1995; Nilsson et al., 1998).

The chamber model is created by fixing two polymethylmethacrylate (PMMA) rings to a microscope slide (also of PMMA), creating two wells. The whole device is coated with heparin to avoid non-specific activation. A second microscope slide (of any [bio]material) is attached, creating two circular chambers that are rotated at 37 °C in a water bath (Hong et al., 1999; Nilsson et al., 1998). After incubation of blood in both models, the attached cells, proteins or activation products can be analyzed.

3.3. Examples of surface modifications to improve blood-compatibility

Materials intended for use in contact with whole blood should ideally be inert with respect to both complement and coagulation activation, allowing blood to pass without any influence by the material. The properties of materials intended for implantation and integration have been carefully investigated for many years and optimized at the material level. However, the biological properties required for tissue integration has not been fully defined.

Heparin coatings have been extensively used to assure the compatibility of biomaterials with blood with regard to activation of the coagulation, contact and complement activation systems. The accepted hypothesis underlying the use of heparin is that down-regulation is achieved by binding regulators such as factor H (Videm et al., 1999), Cl inhibitor and antithrombin. The coagulation system is inhibited by soluble heparin at much lower concentrations than is the complement system (Gong et al., 1996). It is therefore reasonable to assume that the optimal concentration of surface-bound heparin optimized to inhibit activation of the coagulation system is insufficient to down regulate complement. We have demonstrated that the blood compatibility of a surface with regard to coagulation, complement and platelet activation can be improved by increasing the heparin surface concentration. The binding of factor H is not influenced by the increased heparin surface concentration, suggesting that this factor is not the primary regulator of complement on heparin surfaces (Andersson et al., 2003).

Surfaces coated with polyethylene oxide (PEO) have been shown to have low non-specific protein adsorption and thrombogenicity, but unfortunately, these surfaces are also potent complement activators (Amiji and Park, 1992; Hansson et al., 2005; Kidane and Park, 1999).

In two recent papers we employed the strategy involving conjugation of a RCA, in our case factor H, to attenuate complement activation at a surface. In the initial study we demonstrated that factor H, when covalently conjugated to polystyrene with SPDP, could inhibit complement activation in the blood chamber model (Andersson et al., 2001). In a subsequent study we used end group-functionalized PEO (Neff et al., 1999) to specifically bind functionally intact factor H to polystyrene surfaces. Using several whole-blood models, we demonstrated the effectiveness of a hybrid surface in which the coagulation-inert properties of the original PEO were supplemented with a specific complement-inhibitory effect (Andersson et al., 2006).

3.4. Complement activation as a primary event in the biomaterial-induced inflammatory reaction

In the case of a human whole-blood model developed for broadly based studies of the inflammatory reaction, one of our main design principles was to ensure that complement and the other inflammatory systems were kept available for free cross-talk (Mollnes et al., 2002). Such a model is required in order to elucidate which mediators act upstream and which act downstream in the network. More recently, we modified this model to allow us to study the effect induced by the clinically common biomaterial polyvinyl chloride (PVC). By specifically inhibiting complement in this system, we were able to identify complement-dependent and complement-independent reactions triggered in the blood by PVC: First, we demonstrated that PVC-induced CD11b (CR3) expression on granulocytes and monocytes was highly complement-dependent, whereas the release of myeloperoxidase, lactoferrin and thrombospondin was not dependent on complement (Lappegard et al., 2004a). All these reactions were completely attenuated when a heparin-coated (CBAS) PVC tubing was used. Second, we showed that PVC-induced IL-8 production was completely dependent on complement activation via C5a, whereas only half of the MCP-1 generated could be attributed to complement, and the rest was produced in a complement-independent manner. In this system, a heparin coating completely abolished MCP-1 production (Lappegard et al., 2004b). Third, we showed that among the three PVC-induced eicosanoid products leukotriene B4, prostaglandin E2 and thromboxan B2, complement was responsible for only the leukotrien B4 synthesis (Lappegard et al., 2005); again, the heparin coating abolished all eicosanoid production. Thus, the complement inhibitory effect of the heparin coating could not explain all the beneficial effects of this coating on the inflammatory reaction, and these findings underscore the importance of creating surfaces with a broad inhibitory potential. Nevertheless, complement is a major inflammatory system that acts upstream to induce a number of secondary inflammatory mediators, and generating biomaterials that are complement-compatible is therefore of great importance.

4. Complement activation by bio-artificial surfaces

4.1. Examples of extracorporeal circulation

Extracorporeal circulation, the diversion of blood from the circulation of the patient to a device outside the body, is a feature of many treatment modalities, including hemodialysis, hemofiltration, CPB, extracorporeal membrane oxygenation

(ECMO), plasmapheresis, leukapheresis and thrombapheresis. Principally, whole blood is led through the device as unseparated blood or plasma. The plasma is produced either by separating whole blood using a membrane/filter, which also triggers incompatibility reactions, or by centrifugation. Here we will concentrate on hemodialysis and CPB, since the complement-related side effects induced by these treatment modalities include most of those seen with other extracorporeal treatments.

4.2. Hemodialysis

The complement-mediated tissue injury associated with extracorporeal treatment has to a great extent been linked to bioincompatibility reactions. Historically, hemodialysis was associated with severe anaphylactic reactions, and even fatal events were reported. Complement activation was related to the so-called "first-use syndrome," in which the patients experienced an anaphylactoid reaction during the first use of a dialyzer, but this reaction was attenuated with re-use (Chenoweth, 1987). Today these problems are less frequent, but adverse reactions still occur. Of equal concern in uremic patients undergoing maintained hemodialysis is amyloidosis and accelerating arteriosclerosis (Amore and Coppo, 2002). The risk of myocardial infarction in these patients is 5–10 times higher than in healthy individuals (Santoro and Mancini, 2002). Many factors contribute to this increased risk: The uremic condition itself leads to disturbed lipoprotein levels, acidosis, toxins, free radicals and infections, all of which can add to the accelerated arteriosclerosis.

Chronic inflammation, such as that found in patients with rheumatoid arthritis, systemic lupus erythematosus (SLE) or chronic rejection, has been shown to be associated with accelerating arteriosclerosis and cardiovascular disease. It is likely that the chronic whole-body inflammation triggered by hemodialysis is the other major contributor to arteriosclerosis in uremic patients. This inflammation is to a great extent driven by complement activation. The dialysis membranes used today activate complement to varying degrees. Cellulose membranes are strong complement activators, while polymers and blends thereof (such as polyarylethersulfone, polyamide and polyvinylpyrrolidone) are less activating (Deppisch et al., 1998). Activation of complement generates fragments (C3b, iC3b and C3dg) and soluble peptides (C3a and C5a) that bind to the hemodialysis membrane Although they are not covalently bound, the anaphylatoxins C3a and C5a may be passively adsorbed to certain charged surfaces. All these fragments are ligands for receptors on leukocytes (PMNs, monocytes and mast cells) that can trigger inflammation and release proinflammatory cytokines such as IL-1β, TNF-α, IL-6 and the highly potent chemokine IL-8.

Another effect of complement activation is the up regulation of receptors on leukocytes (e.g., CD11b/CD 18 and CD35), which together with down-regulation of L-selectin makes the cells very adhesive and prone to interact with platelets and endothelial cells in the vascular walls (Moen et al., 1997; Nilsson et al., 1998). This cellular activation also leads to increased levels of acute-phase proteins such as CRP, allowing these molecules to bind to activated endothelial cells and activate complement. CRP and complement components such as C4, C3 and C9 are present in arteriosclerotic plaques together with monocytes/macrophages (Torzewski et al., 2000; Vlaicu et al., 1985).

4.3. Cardiopulmonary bypass (CPB)

CPB procedures have increased dramatically over the past decade as a result of vascular bypass surgery. The CPB procedure is, however, associated with a number of side effects related to the attendant trauma, the contact made by the blood with the material surface and the gas surface, the neutralization of the heparin by protamine, and the ischemia/reperfusion triggered by the blood in the bypassed tissues (lungs and heart). The resulting cellular and humoral defense reactions are termed the systemic inflammatory response syndrome (Bown et al., 2003). Included in this syndrome are hemostatic disorders that take the form of bleeding, platelet activation and the generation of macro- and microthrombi. The consequence of these disorders is a high incidence of thrombotic events, including postoperative myocardial infarction and stroke that can produce serious neurological symptoms. Recent studies have shown that the incidence of stroke is in the range of 1–4% among CPB patients (Nussmeier, 2002). Another neurological problem often associated with CPB is prolonged cognitive dysfunction, which may be caused by microthrombi (Mojcik and Levy, 2001). Many of these problems result from complement activation, an effect that is supported by in vivo studies using anti-C5 antibodies in which complement inhibition was found to attenuate postoperative myocardial injury, cognitive deficits and blood loss (Fitch et al., 1999).

Complement activation occurs on both the biomaterial surfaces exposed in the extracorporeal circuit and on the gas surface (Pekna et al., 1993) that is exposed to the blood in the oxygenator of the CPB circuit. Complement is also activated by the platelets in the clotting blood (Ozmen et al., 2002), and a final spike is generated when soluble heparin is neutralized with protamine (Fosse et al., 1997). Together, these processes create a period of complement activation that lasts for at least the duration of the CPB procedure. Complement affects both leukocytes and platelets. In an in vitro study, Rinder et al. (1995) showed that platelets expose P-selectin and that platelet/leukocyte (PMNs) complexes are generated during CPB. Both these phenomena are totally abrogated by anti-C5 antibodies and are likely to contribute to the disturbed hemostatic balance during CPB.

Complement activation products, in particular C5a, rapidly up-regulate the expression of CD11b/CD18 and CD35 on PMNs and of C3a on monocytes (Rinder et al., 1999). Other signs of an inflammatory response are the release of cyto- and chemokines and their contribution to an acute phase reaction. Hyporeactivity against various pathogens have been demonstrated in vitro after CPB, and it is likely that this extensive inflammatory response may explain the increased risk of post-operative infections (Hill et al., 1997; Marie et al., 1998). Modification of the surface by the addition of a heparin coating has improved the biocompatibility of extracorporeal devices (Hsu, 2001). Although initially intended to reduce the requirements for systemic heparinization, heparin coating has also been shown to markedly

attenuate inflammatory reactions, including the activation of complement.

In the tissues that are removed from the circulation during CPB, the lungs and heart, ischemia occurs slowly. Complement activation is triggered by the ischemia itself and particularly by the reperfusion of blood through the organs. Several mechanisms can induce the activation of complement during reperfusion: Naturally occurring IgM antibodies recognizing neoantigens exposed on damaged endothelial cells may activate the CP (Carroll and Holers, 2005). MBL may recognize carbohydrates expressed on the damaged endothelial cells, and subsequent deposition of C5b-9 may enhance the cell damage (Collard et al., 2000). This process is consistent with the observation that in humans undergoing a major reperfusion after repair surgery for a thoracoabdominal aortic aneurysm, the complement system is activated in an MBL-dependent manner (Fiane et al., 2003). C5b-9 also activates the endothelial cells, thereby stimulating tissue factor expression and up-regulation of E-selectin and ICAM-1 (Kilgore et al., 1995; Tedesco et al., 1997). Combined with the binding of iC3b to the surface of the endothelial cells, PMNs and monocytes already activated by the CPB procedure can adhere to the endothelial cells and migrate into the tissue contributing to the inflammation. The post-bypass syndrome, characterized by symptoms of hypotension and dyspnea, may arise directly from this mechanism.

5. Pharmacological complement inhibition

Several pharmacological agents that regulate or modulate complement activity have been identified in in vitro assays, but to date there are no specific inhibitors of complement activation in routine clinical use. However, several recombinant protein candidates are under development: One is a recombinant form of complement receptor 1 known as soluble complement receptor 1 (sCR1; TP-10). This C3 convertase blocking agent was first shown to markedly reduce experimental myocardial infarction and is now in clinical trial (Keshavjee et al., 2005; Weisman et al., 1990). Another is a humanized monoclonal anti-C5 antibody (pexelizumab, eculizumab) that blocks the activation of C5 (Kaplan, 2002). Thus, both inhibitors prevent the formation of C5a and C5b-9, but the anti-C5 antibody does not affect the activation of C3, suggesting that the unwanted effects of C3 activation are not affected but the main antimicrobial defense related to C3 is preserved. Both derivatives have been tested in in vivo animal models of disease and in patient clinical trials, and an efficient inhibition of complement has been confirmed (Keshavjee et al., 2005; Hill et al., 2006). A conjugate of sCR1 and the selectin ligand sLe(X), combining inhibition of complement and cell adhesion, was found to be even more efficient than sCRl alone (Huang et al., 1999). Use of a shorter, membrane-targeted form of sCRl (APT070) has also been used in a surfacetarget-bound approach to inhibit complement activation (Smith, 2001). Attention has also been paid to recombinant forms of DAF and MCP, a conjugate of these two, the complement activation blocker-2 (CAB-2; Higgins et al., 1997) and membrane-targeted CD59 (Hill et al., 2006). However, none of these reagents has yet reached clinical trials. Because of their large molecular size (240 and 26 kDa [scFv fragment], respectively), soluble CR1 and the anti-C5 antibody have the disadvantage that they must be administered parenterally. Emphasis is now being placed on developing smaller active versions of these molecule with longer half lives and on decreasing their cost. These considerations are most important for clinical applications that require relatively long-term administration.

We and others have identified active sites on complement proteins and used synthetic peptides based on these sites as inhibitors for complement activation. However, this approach has not yet yielded a potent (at least equimolar to the protein of interest) peptide that can specifically inhibit each of the initial pathways of complement activation. During the past decade we have identified and characterized a peptide that binds to C3 and prevents its further activation by the CP, LP and AP (Sahu et al., 1996, 2000). This complement-inhibiting peptide, Compstatin, is a disulfide-bonded cyclic peptide of 1550 Da. We have demonstrated that this peptide is significantly active both in vitro in the tubing loop system (Nilsson et al., 1998) and ex vivo in a perfused xenograft system (Fiane et al., 1999). In a subsequent study, we demonstrated that *N*-acetylated Compstatin at less than a six-fold molecular excess over C3, was able to inhibit complement activation totally in vivo in a baboon heparin/protamine complement activation model (Soulika et al., 2000). Complement inhibition was accomplished, without adverse effect, by the injection of Compstatin at 25 mg/kg, a dose level that is comparable to those of other bioactive peptides such as relaxin (80–100 mg/kg) and cyclosporin (10 mg/kg). Various combinatorial and rational design approaches led to the generation of a novel Compstatin analog that is about 260 times more active than the original Compstatin (Katragadda et al., 2006); experiments are in progress to assess the in vivo efficacy of this compound.

C5aR agonist peptides have been available for a number of years, but the search for a C5aR antagonist peptide has only recently been successful (reviewed in Morikis and Lambris, 2001)). The design of a C5a agonist started with site-directed mutagenesis within the C-terminal helix of C5a (Mollison et al., 1989) and subsequent design based on truncated peptides spanning the 10–6 last residues of the C-terminal helix (Ember et al., 1992; Kawai et al., 1991; Tempero et al., 1997). This approach, in addition to C5aR agonists, yielded peptides that showed inhibition of C5aR binding (Konteatis et al., 1994). Subsequent studies using rational design and structure determination by NMR resulted in a number of C5aR antagonists that exhibited small variations in sequence and structure (Fitch et al., 1999; Gerber et al., 2001; Wong et al., 1998). The most potent C5aR antagonists have shown activity in several models in vitro and in vivo (Arumugam et al., 2002; Finch et al., 1999; Haynes et al., 2000; Mastellos et al., 2001; Mollnes et al., 2002; Short et al., 1999; Strachan et al., 2001; Wong et al., 1998; Woodruff et al., 2001), including the expression of β2 integrins (Mollnes et al., 2002), production of inflammatory cytokines (Lappegard et al., 2004b), liver regeneration (Strey et al., 2002), sepsis after cecal ligation/puncture (Huber-Lang et al., 2002), antiphospholipid antibody-induced fetal loss (Girardi et al., 2002), mesenteric ischemia/reperfusion-induced injury (Fleming et al., 2002); choroidal neovascularization (Nozaki et al., 2006), and

superantigen-induced immune complex-mediated inflammation (Anderson et al., 2006), lupus nephritis (Bao et al., 2005), and artificial surface-induced eicosanoid production (Lappegard et al., 2005).

6. Conclusions

The clinical consequences of complement-associated bioincompatibility reactions during extracorporeal circulations call for an increased knowledge of how complement activation is triggered by the biomaterial surface, as a means for developing materials with lower or no complement-activating properties. Given the complexity of the situation, with multiple interactions occurring among the various cascade systems and cellular systems of the blood, full protection will most likely require the additional use of soluble complement inhibitors. The inhibitors under development today (i.e., recombinant proteins) will be very expensive to use on a daily basis for applications such as the treatment of uremic patients. Therefore, it is important to develop simple peptide-based inhibitors like Compstatin for use as low-cost complement inhibitors in clinical applications.

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